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PREPARATION AND CHARACTERIZATION OF CONDUCTIVE SCAFFOLDS FOR NEURAL TISSUE ENGINEERING

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ABSTRACT

Damages to the nervous system are one of the health challenges. Using electrical stimuli because of the nervous system's electrical nature has been introduced as a solution to differentiate the stem cells successfully in recent years. Electrical stimulation (ES) has been used in various cell culture methods to grow stem cells such as nerve stem cells (NSCs), nerve differentiation, migration, and repair. Electrical stimulation mechanisms direct axon and neurite growth and cause directional cell migration, while magnetic fields cause neurogenesis and help NSC differentiate into functional neurons/nerve cells. Conductive nanomaterials have been utilized as functional scaffolds to provide mechanical support and biophysical signals to direct the growth and differentiation of nerve cells and form complex neural tissue patterns. Electrical signals may improve stem cell neurogenesis through activating specific ion channels, such as SCN1α. This article can be used as a checklist for ES work in stem cell research to expand using stem cells in clinical applications. This study revealed that electrical conductive materials and applying electrical and magnetic signals to stem cells could be a promising option in treating diseases of the nervous system, such as spinal cord injuries. , Parkinson's, and other diseases related to the nervous system.

KEYWORDS: Central Nervous System (CNS), Electrical Stimulation (ES), Electric Fields (EFs), Electromagnetic Fields (EMFs), Conductors, Conductive Polymers.

INTRODUCTION:

Nerve tissue has been expanded throughout the body in the form of a network of integrated communications. Anatomically, the nervous system is organized wholly in two main parts: the central nervous system (CNS, which involves the brain and spinal cord), the peripheral nervous system (PNS), which directs sensory impulses central nervous system and carries motor impulses to the environment. This system is made of cerebral, spinal, peripheral nerves, and ganglia (named small clusters of nerve cells outside the CNS).

Nerve stem cells (NSCs) are self-replicating stem cells with the multifaceted capacity to produce neurons and glial cells in the nervous system. NSCs are the most promising option in tissue engineering and regenerative medicine because of their tissue formation and regenerative capacity at nerve damage sites.

In recent decades, researchers have been increasingly interested in conducting various research on developing smart materials. Such cases have been usually designed for external stimuli (physical, chemical, mechanical) and behavior similar to the body's natural tissues. The piezoelectric scaffold can be named as such a type of intelligent material, which can produce electrical signals as a result of applied stress. Also, they can stimulate signaling pathways, increase tissue regeneration at the injured site. This is particularly applied to nerve tissue, where electrical charges are essential for cellular activity. A wide range of synthetic and natural polymers have been investigated as electrospun scaffolds for neural tissue engineering. Nerve regeneration is a complicated and local biological phenomenon that causes it challenging to heal patients with nervous system disorders.

MECHANICAL AND PIEZOELECTRIC TRANSMISSION IN LIVING ORGANISMS:

Mechanical transmission is one of the different ways in which cells can sense and convert mechanical stimuli into electrochemical activity. The extracellular matrix and s.c cells are the best recognized direct element in the pathway between external transmitted stress. Stress activates channels. Voltage-dependent channels are usually specific to one type of ion, for example, sodium (Na +), potassium (K +), calcium (Ca +), and chloride (Cl-) ions. Natural piezoelectricity of the body's body elements provides the electrical charges in response to mechanical stress in voltage-dependent channels. [5]

UTILIZATION OF PIEZOELECTRIC BIOMATERIALS IN NEURAL TISSUE ENGINEERING:

Piezoceramics are the first group of studied piezoelectric materials. The rat's cortical neurons are cultured on pzt with a poly-L-lysine coating and grow longer axons, notwithstanding reducing the cell numbers. Besides, the frequency and amplitude of stimulatory postsynaptic currents increase, symbolizing that piezoelectric property can perform more neural activity. Aw should consider that they are applied for medical applications, principally actuators, converters, and sensors. It is not applied in the pure solution for medical implants because of allergic reactions. Advanced medical applied systems are composites based on polymer matrices with ceramic fillers in the form of fibers. Lopez and et al. convert barium nanoparticles into a polymer matrix to repair bone defects, which produces approximately high polarization. Also, this system decreases vulnerability and can be exercised as an electroactive scaffold.

PVDF has been largely investigated among the types of piezoelectric polymers; it is essentially because of its high piezoelectric power, processing, good chemical resistance, temperature stability, and excellent mechanical properties against other piezoelectric polymers. PVDF is possible to be available in at least five crystalline polymorphic phases, in which the beta phase shows the highest piezoelectricity that reaches 20 pC / N.

Using PVDF in piezoelectric scaffolds in tissue engineering requires applying fabrication techniques that allow the proper shape to be formed, and the polar phase content is responsible for the high piezoelectricity. The electrospinning technique is one of the promising fabrication techniques that satisfy both expectations. Many papers are about the electrospinning of PVDF nanofibers in the solution. The importance of the effect of processing parameters on nanofibers' structure and properties and the properties of nonwoven nanofibers of PVDF beta phase content have been studied in terms of applying voltage and rotational speed of the rotating collector. This collector's rotational speed is related to the mechanical deformation that causes to form the polar phase. Liu et al. formed nanofibers with various rotational speeds from the collector: 900, 1100, 1300, 1500, 1700, and 1900 rpm. XRD diffraction indicated a peak around 20.6-20.9 deg, which belonged to the beta phase, but the alpha phase peak had vanished. They received PVDF piezoelectric fibers with smaller radii, a smooth surface shape, and a fitting beta phase at 1900 rpm. According to new research, increasing the speed of the rotation of the collector will increase the beta phase of the beta piezoelectric content.

It has been shown that long-term utilization of piezoelectric stimuli to neurons increases the number, length, and branching of nerve cells. There was no effect on neurite regeneration when vibrations were applied to non-piezoelectric materials. This supplemental polymer has been explained with the highest electroactive properties among the piezoelectric materials with piezoelectric power up to 30pC / N. PVDF-TrFE creates the beta phase form co-polymerization without using mechanical tension. If more bonding is needed, mechanical tension, electrical polarity, it is conceivable to increase the crystallinity and adjustment of the CF2 dipoles. Accordingly, depending on the TrFE content, it is possible to induce more piezoelectric power against PVDF. Electrospun PVDF-TrFE fiber scaffolds exhibited higher crystallinity and beta phase content compared to neuromuscular engineering raw material powder. It has been announced that the piezoelectric membrane mixture of PVDF-TrFE with barium titanate can induce bone regeneration by producing a charge. PVDF-TrFE piezoelectric fiber scaffolds have been employed to analyze their effect on nerve repair. Numerous studies have reported the positive impact of these scaffolds on the growth and differentiation of nerve cells. Lee et al. designed a PVDF-TrFE piezoelectric electrospun scaffold with various arrangements of fibers in a scattered and parallel form. And it was shown that scaffolds with parallel fibers have the most magnificent potential to be used in neural tissue engineering. PVDF films affect cell proliferation in the regeneration of loading muscle cells on the plate. Nevertheless, a study with specific dynamic conditions

for PVDF-TrFE piezoelectrics has not been conducted so far with mechanical or electrical actuators.

PHBV is polyester with low piezoelectric strength (1.2 pC / N). It is a thermoplastic constructed by many bacteria as a source of carbon storage and intracellular energy. The polyester is also biodegradable and biocompatible, with strong mechanical properties that enable us to use PHBV as a scaffold and biosensor. PHBV has a bone-related piezoelectric power that can improve bone growth and development. It can further be utilized in association with hydroxyapatite to bone tissue engineering. PBHV has been studied for nerve tissue engineering for nerve cell growth and polarization of dendrites. PBHV electrospun fibers can be applied for neural tissue engineering by adding collagen.

Poly-L-Lactic Acid is a biodegradable, biocompatible polymer with a piezoelectric power of -10pC / N. Foucada et al. have explained that applying PLLA can grow the bone in response to its piezoelectric polarity. It is possible that PLLA has some applications with a structure similar to the extracellular matrix (ECM) in biomedicine. Lateral nanofiber scaffolds coated with graphene oxide increase cell growth. Ultimately, adding iron nanoparticles supports the proliferation of neurites along with the PLLA microfibers.

Cellulose with piezoelectric strength of 0.1 Pc / N has been largely investigated among natural polymers. Cellulose is a linear homopolymer of glucose or high biocompatibility. Cellulose is utilized in various shapes and designs: membrane sponges, microspheres, and woven or non-woven textiles.

Regulating migration and its rate is different according to the electric field's amount and frequency and the cell source and culture conditions. Intracellular proteins and chemokines related to cell attachment, migration, and cytoskeletal rearrangement are more noticeable than PI3P and Rho GTPase / Rac attack but not CXCR4 in the presence of EF. Activation of the intercellular signaling pathway is possible to be related to the NSC source and the ES program. Researchers are developing EFs' understanding of the fate of NCS by advancing biomaterials to control the fate of NCSs after injury. The final objective is not to apply these cell-based scaffolds to repair nerves, but most of the material has previously been assessed in the culture, and even a few scaffolds have been tested in vivo on animals. There are four types of structures that have been analyzed, including foams, hydrogels, electrospun fibers, and thin films. Extremely porous foam scaffolds are largely composed of graphene, which is conductive. It has been proved that graphite scaffolds support NCS attachment, viability, neuronal transformation, stellar cells, and oligodendrocytes in both two and three dimensions. When an electric current is applied to graphene, NCSs are converted much more to neurons and much fewer star cells in culturing graphene scaffolds and nanonetworks. Furthermore, polyethylene glycol (PEG) hydrogels with polyaniline (PANi) or carbon nanotubes produced more transformed neurons with increased nutrient proliferation. PANi and carbon and PPy nanotubes have also been used to produce conductive fibers and thin films to clean neurons and stimulate NCSs. Among these materials, PPy thin films have been applied for electrical conditions before implementation. After transplantation, NCSs increased the production of growth coefficients, which resulted in angiogenesis and enhanced post-stroke functional outcomes (George et al.). Since many of these substances have the inherent ability to improve neuronal transformation and growth without EF, delivery of NSC-based and ES scaffolds post-traumatic injuries to promote repair is expected to have a brilliant future.

Table 1: Electric fields can lead to changes in NCSs. Including behavioral changes, phenotypic, and destiny selection responses. The NSC's response to ES depends on the source of the NSC, the sub-layer, and the stimulation program. As specified below. [4].

Main results	Excitation Parameters	Sub-layer	NEC source
ES increased neuronal transformation ES increased the flow of Ca2 + inward • Neuronal phenotypes were observed after transplantation of pre-stimulated cells	DC, percussion V = 0-20V / 4mm tPulse = 5at950µs intervals	PDL with TCP coverage	The fetus Mice, E3.5 Cell line: R1
ES led to galvanotaxis toward the cathode ES-dependent effect of strength on direction and speed • Requires NMDRAs, which actin increased Rho GTPase RAC1	DC V=0-250 mV/mm t_{DC}=3hr $t_{Post-ES}$=12-13hr	PLL-LN with TCP coverage	Ventricular Rat, E17-18 Main
ES led to galvanotaxis toward the cathode ES led to lamelipods toward the cathode • .Without EF, NCSs have the same metabolic activity as amoebas PI3K inhibitor reduced cathodic bias •	DC V=250mV/mm t_{DC}=145min	PLO-LN with TCP coverage	Hippocampus Rats, adults Cell line: HCN-A94
(Hz: Neural <Stellar Cell Transformation (DC0.1 (Hz: Neural> Stellar Cell Transformation (DC1 • mV / mm, 0.1Hz and 2mV / mm, 1Hz increased the percentage of transformed cells 4 • .((DC (Hz survival was best (AC1 • Hz, 16mV / mm: Neural <21 days (AC) metrocytic transformation0.1 (Hz Neuron <Transformation of Stellar Cells (AC1 •	DC V=2-16mV/mm f=0.1-10Hz t_{DC}=0-21d AC V=2-16mV/mm f=0.1-10Hz t_{DC}=1-21d	Alginate beads	[Front of the midbrain [former midbrain Mice, E14 Main
(Cells arranged perpendicular to the EF vector (AC • (Increased neuronal transformation without increasing glia cells (DC • Expanded perpendicular nutrient propagation for EF (DC) increases (Decreased cell count due to viability (DC • (No difference in orientation, transformation, multiplication (AC •	DC V=437mV/mm I=3.4mA tES=4d AC V=46mV/mm I=0.338mA f=0.5Hz tES=4d	Glass with PLL-LN coating	Hippocampus Rats, adults Donation source
ES increased proliferation • ES metamorphosed neurons at 4µA for 200µs Metamorphic cells were not evaluated	DC, beat I = 4-31µA / cm2 f = 100Hz tPulse = 50.200µs	Glass with tin oxide and indium coating	the brain Mice, E13 Main
Neural cues migrated to the cathode • ES increased galvanotaxis via PI3K and Akt ES Galvanotaxis increased NPCs in the organotypic model ES increased PIPP3 levels, leading to the reorientation of surface sensors and the actin cytoskeleton Galvanotaxis requires FGF and EGF	DC V=0-500mV/mm t_{DC}=5hr	TCP with PLO-LN coating, spinal incision	Hippocampus Mice, adults Cell line: HCN-A94 the brain Rodent, E12-E14 (Main, WT (rat (Or PI3K - / - (mouse

ES increased proliferation ▪ ES increased the spread of nitrite on biomaterials	DC V=100mV/mm t_{ES}=1hr	PLLA and PANi Electrospun nanofibers	Cerebellum (Rat (infant Cell line C17.2
ES led to galvanotaxis toward the cathode Galvanotaxis was time and voltage dependent .ROCK inhibition did not affect orientation .CRCX4 inhibition did not affect galvanotaxis	DC V=16-300mV/mm t_{ES}=1hr	TCP with PLO-LN coating	hESC Human, embryonic Cell line: C17.2
CNT cords support adhesion, proliferation, neural sphere formation, and NSC nutrient proliferation .10mV reduced viability after nine days ▪ 5mV increased volatility and neutrite migration ▪ .CNT + ES increases ENO2 and MECP2 in one week, not two weeks .CNT + ES increases TUJ1 and MAP2 in three weeks and decreases nestin	DC pulse V=0-10mV I=0.5mA t_{Pulse}=25ms t_{ES}=15min/d; 9d	Carbon nanotube ropes	Hippocampus Rats, adults Cell line: HCN-A94
Materials were compatible with reproduction ▪ Stimulation with flash light increased proliferation .Flash stimulation increased metamorphic neurons Stimulation with flash light reduced star cells .There are directional currents between the realm and the neuronalization sites .Cells migrate to the olfactory bubble at the cathode .The direction and rate of migration depends on the voltage ▪ P2Y1 promotes targeted migration ▪	Stimulus: Light flash mW / cm210 F = 1Hz tPulse = 4s, 30min / 12hr DC V = 3-250mV / mm I = 1.5µA / cm2	Graphene nanoparticles	the brain Man Cell line
.There are directional currents between the realm and the neuronalization sites .Cells migrate to the olfactory bubble at the cathode .The direction and rate of migration depends on the voltage ▪	DC V=3-250mV/mm I=1.5µA/cm^2	Tissue cutting	Ventricular Mice, adults Main
P2Y1 promotes targeted migration ▪			
.Both two-dimensional and three-dimensional support NSC growth D: Increased duplication (Ki67) on 2D movies3 Three-dimensional: Increased differentiation of neurons and star cells compared to two-dimensional ES increases the uptake of Ca2 + into NSC-derived neurons	DC, cathodic pulse I = 20-30µA tPulse = 1-100ms, 10s	LN coating: 3D graphene foam, 2D graphene thin layer	Hippocampus Mice, P1 Main
.There is no evidence of orientation in EFs ▪ (Nestin was paid in D3 and D7 and was not seen in D14 (changing ▪ Increases in GFAP and Tuj1 were observed at 10Hz and 50Hz (late maturation of star (cells	AC V=100mV/mm f=1-50Hz t_{ES}=4hr/d 3,7,14d	Electrode with PLL coating	Cortex E45 Yucatan Pig Main
ES increased neuronal transformation and neutrophil length .Intracellular Ca2 + stimulation increased	DC V=0.53, 1.83mV/mm t_{ES}=10min/d, 2d	PDL-LN glass	Underwater Rats, adults Main
Survival and metamorphism of scaffold support cells ▪ .Cells exhibited Ca2 + signaling on SWNT-PLGA, but did not on PLGA-only scaffolds ES leads to an increase in the activity of Ca2 + and altered neurons	DC I=30µA t=10min	Electrical scaffolds SWNT-PLGA	iPSCs Man Cell line: HFF1
Nervous leaders migrated to the cathode ▪ .A stronger effect was seen at 100mV / mm than at 50 ▪	DC V=0-100mV/mm t_{ES}=20 hr ES	Embryo-like bodies Mice, embryos Cell line: ES-D3	Embryo-like bodies Mice, embryos Cell line: ES-D3
.Two-phase monopolar ES increased migration in unchanged (cathodic) NPCs .Two-phase bipolar ES and the absence of ES lead to undirected migration .Transformed NPCs do not migrate under ES ▪	AC, 5V Cathodic ;unipolar 5ms 1.25V anode; 20ms AC, 5V Cathode ;bipolar 5ms 5V anode; 5ms	Torque with matrigel-PLL coating	Ventricular Mice, adults Main
.Cells migrated to the cathode Detention of Wnt and GSK3β reduced ES migration ▪ Asymmetric redistribution of CLASP2 ▪	DC V=0-400mV/mm t_{ES}=2-6hr	TCP with PDL-LN coating	In front of the midbrain Mice, E12.5 Main
(+ ES increased metamorphosis (Tuj1 +), decreased glial metamorphosis (GFAP ▪ ES increased cell clustering and nutrient proliferation	AC I/A=0.25mA/cm^2 t_{Pulse}=100/20µs t_{ES}=8hr/d (3d)	PPy	the brain Human, embryonic Cell line: ReNcell CX
ES led to Ca2 + -dependent galvanotaxis .Cathode cell migration was 72% ES versus 18% non-ES ES increases the transformation of NPCs into neurons, but does not for glia cells. (10% (to 20% in most strains of astrocytes	DC V=115mV/mm t_{ES}=1.5hr, 2hr/d $t_{Post\ ES}$=9d	Glass with PDL-matrix coating	Ventricular Mice P2-P7 Main
ES increased the length of the main nutrient and branching process. Transformation into neurons, stellate cells, and oligos. ES increased transformed cells with more neurons in 7 days. ES increased oligodendrocytes at 7 days and 14 days	DC, percussion V = 300mV / mm f = 100Hz tES = 48hr tPostES = 0.411d	Glass with PLL coating	the brain Mice, E13.5 Main

ES increased cell density on days 5 and 7 PANI and ES had no effect on viability ▪ ES increases nutrient volatility ▪ ES increased β-III, BFABP, PMP22 transcripts and decreased gene expression and ICC. No trace from PANI	AC V=0-75mV, f=200Hz t_{on}=6hr/d (1,3,5,7d)	Films PEG-PANI	Not reported Man Cell line
ES increases migration distance in hNPCs Migration in 10 days> Migration in one day ▪ Blocked calcium channels by galvanotaxis No change due to ES Migration to the Andes ▪	DC, pulsedV=250mV/mm I=0.1-1.5mA f=1-1000Hz $t_{on/off}$=0.045-4.5s t_{on}=6hr	TCPS	the brain Human, embryonic Cell line: ReNcell CX

CONCLUSION:

The use of stem cells presents challenges. One of these challenges is the successful differentiation of stem cells towards the specific functional neurons. In recent years, various researches have shown that the use of electrical stimulating agents and conductor substrates can be used as a suitable way to successfully differentiate stem cells toward neurons because of the nervous system's electrical nature. Electrical stimulation (ES) has been used in various cell culture methods to grow stem cells such as nerve stem cells (NSC) neural differentiation, migration, and repair. Electrical stimulation mechanisms guide the growth of axons and neurite and cause directional cell migration, while magnetic fields cause neurogenesis and facilitate NSC for differentiation into functional neurons. In this research, different methods of electrical stimulation and conductor substrates for successful differentiation of stem cells and correct behavior of stem cells have been investigated.

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